BPSA: A Novel Serum Marker for Benign Prostatic Hyperplasia

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Free prostate-specific antigen (fPSA) testing was developed and approved for widespread use despite the lack of knowledge regarding the underlying biologic basis for its ability to discriminate between benign prostatic hyperplasia (BPH) and prostate cancer. We hypothesized that the relationship of total PSA to prostate volume was due primarily to the fPSA component of serum PSA, and we studied the molecular forms of PSA found in prostate tissue. Later, more sophisticated studies resulted in the discovery of BPSA (benign PSA), a novel form of fPSA associated with nodular hyperplasia of the transition zone (TZ). We found that the serum BPSA level is highly correlated with TZ and total prostate volume. In our most recent studies, we found that BPSA correlates better with TZ volume than does PSA and can predict clinically significant prostate enlargement better than PSA or fPSA. Furthermore, the relation of BPSA and fPSA to total prostate and TZ volumes is independent of age.

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It is undisputable that the discovery and widespread use of prostate-specific antigen (PSA) as a screening test for prostate cancer has revolutionized the clinical practice of urology. Despite passionate debate, PSA-based screening programs have clearly had an impact on reducing mortality due to prostate cancer, as evidenced by SEER (Surveillance, Epidemiology, and End Results) data from the United States[1] and most dramatically in the population-based experiment performed

in Tyrol, Austria.[2] PSA measurement has dramatically improved our ability to more accurately stage newly diagnosed prostate cancer and has been the mainstay in predicting outcomes using various risk-stratification schema, such as the Partin tables,[3] or continuous nomogram-based models.[4] Finally, PSA measurement has been an indispensable tool for assessing the success of prostate cancer therapy and monitoring patients for evidence of disease progression.

As significant as these accomplishments are, PSA has influenced medicine in many other profound ways. For example, PSA basic research has been a paradigm for the "bench-to-bedside" notion of translational research—that is, basic laboratory research that is quickly translated into clinically relevant changes in patient care. For example, the basic research discoveries of Stenman and colleagues[5] and Lilja and colleagues[6] that PSA exists in multiple molecular forms in the blood—namely, "free" or uncomplexed forms (fPSA) and forms complexed to protease inhibitors such as antichymotrypsin (ACT)—were quickly transformed into clinically important and useful science by the further observations that the ratio of these forms of PSA were associated with the presence of prostate cancer in patients with modest elevations in total PSA levels. These observations, followed by the innovation and hard work of many other investigators, culminated in Food and Drug Administration (FDA) approval of percent free PSA (% fPSA) as a marker to help differentiate men with benign prostatic hyperplasia (BPH) from those with prostate cancer when PSA is in the range of 4 ng/mL to 10 ng/mL.[7]

The FDA approval of fPSA was remarkable in the sense that it was clinically developed and approved for widespread use despite the lack of knowledge regarding the underlying

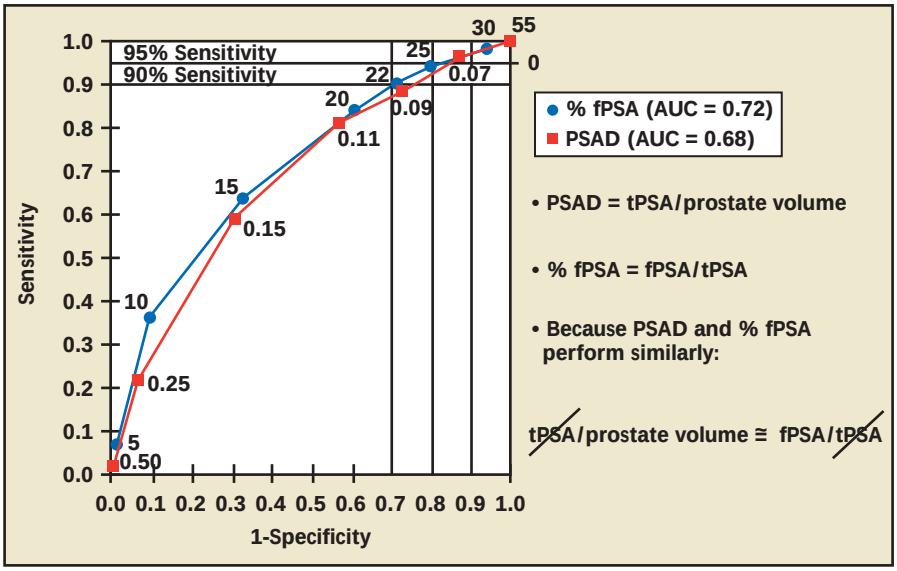

Figure 1. *Percent free prostate-specific antigen (% fPSA) and prostate-specific antigen density (PSAD) receiver operator characteristic curves. tPSA, total PSA; AUC, area under the curve. Adapted from Catalona WJ et al.* Urology. *2000;56:255-260.*[9]

biologic basis for its ability to discriminate between BPH and prostate cancer. One early theory proposed that BPH tissue fails to locally produce ACT, which is therefore not available to complex with PSA, in contrast with prostate cancer, in which this protease inhibitor is abundantly present.[8] However, although neither this theory nor any other was convincingly proved, acceptance and use of fPSA steadily increased among urologists and even primary care physicians.

At Baylor College of Medicine, a confluence of work in our laboratory, in collaboration with others, shed some light on this perplexing biologic conundrum. For example, in a study published in 1994, we showed that, utilizing data from the 7-center pivotal trial for fPSA FDA approval, receiver operator characteristic (ROC) curves evaluating the performance of both % fPSA and PSA density (PSAD) were almost identically overlapping (Figure 1).[9] Therefore, we surmised that if PSAD = total PSA (tPSA)/prostate volume and % fPSA = fPSA/tPSA, and because PSAD and % fPSA performed similarly, then PSAD $\cong$ % fPSA, tPSA/prostate volume $\cong$ fPSA/tPSA, and after some simple algebraic reduction of equations:

absolute fPSA $\cong$ prostate volume

PSA and Prostate Volume

These were not the first indications that PSA was strongly tied to prostate volume. In 1990, Babaian and colleagues[10] observed that serum PSA level was associated with ultrasound-measured volume of the prostate in men with negative prostate biopsy. The premise of PSA density was based on the notion that by "correcting" serum PSA levels for the presence of BPH, one could more accurately assess the risk that elevated PSA levels might be due to prostate cancer.[11] Roehrborn and colleagues[12] further explored this relationship, demonstrating for the first time that serum PSA levels were highly correlated with prostate volume in an age-dependent manner; that is, the slope of the rise in serum PSA levels with higher prostate volume became steeper with age.

Although the notion that serum PSA levels reflect the presence of

BPH in men without prostate cancer was important, Noguchi and colleagues[13] took these ideas to a new, more controversial level when they proposed that, even in men with localized prostate cancer, serum PSA levels were primarily reflective of coexistent BPH and had little, if anything, to do with prostate cancer. These provocative conclusions came at a time when an increasing amount of evidence was accumulating that PSA level is a poor indicator of the presence or biologic potential of prostate cancer. In this climate, we hypothesized that the relationship of tPSA and prostate volume was due primarily to the fPSA component of serum PSA, and we set out to further explore this relationship.

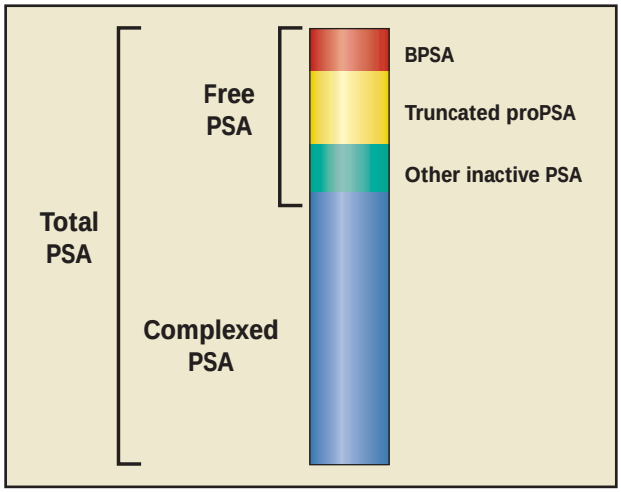

Figure 2. *Heterogeneity of serum prostate-specific antigen (PSA). BPSA, benign PSA.*

Discovery of BPSA and proPSA Isoforms

In response to these quandaries, our laboratory began to study the molecular forms of PSA found in prostate tissue harvested at radical prostatectomy from 3 clinically important yet different areas of the prostate: noncancerous peripheral zone, peripheral zone cancer, and benign transition zone (TZ) of the prostate.[14,15] Our early studies focused on quantifying, using Western blot analysis, the levels of fPSA, complexed PSA, and ACT in these areas of the prostate. We hypothesized that the forms of PSA found in prostate tissue, which are present in milligram-per-milliliter quantities and thus much easier to study, would reflect the character of PSA found in serum at nanogram-per-milliliter quantities. Later, in close collaboration with researchers from Hybritech, Inc (now a subsidiary of Beckman Coulter, Inc., Fullerton, CA), more sophisticated studies using affinity columns and hydrophobic interaction column chromatography culminated in the discovery of BPSA (benign PSA), a novel form of fPSA associated with nodular hyperplasia of the TZ.[16] These studies also demonstrated a clear association of truncated molecular forms of proPSA with the prostate peripheral zone, including prostate cancer.[17] More recent studies using serum assays specific for these various molecular forms of fPSA have demonstrated that the majority of fPSA in the blood is composed of BPSA, truncated forms of proPSA, and an additional form of intact yet inactive PSA (Figure 2).

The median BPSA level in patients with symptomatic BPH was significantly higher than in patients without BPH symptoms.

Our studies demonstrated that BPSA is predominantly clipped at amino acid residues lysine 145-146 and lysine 182-183 and is elevated in the TZ epithelium of prostates with nodular BPH (Figure 3).[16] More recent studies have shown that BPSA is also present in seminal plasma.[18] A dual monoclonal antibody assay for BPSA (detection limit of 0.06 ng/mL) has been evaluated in men with symptomatic BPH, men without clinical BPH, and healthy control subjects. The median BPSA level in patients with symptomatic BPH was significantly higher than in patients without BPH symptoms. In the healthy control group, BPSA was almost undetectable.[19]

In a soon-to-be-published study, we found serum BPSA level to be highly correlated with TZ and total prostate volumes. Because fPSA is composed of multiple distinct molecular forms of PSA that can originate from cancer, benign peripheral and TZ tissues, and BPH-associated nodular hyperplastic TZ tissue, we hypothesized that BPSA would outperform both total PSA and fPSA as a predictor of prostate enlargement. Autopsy studies suggested that age-related increases in prostate volume occur via 2 distinct processes: enlargement of BPH nodules within the TZ of the prostate (nodular BPH) and diffuse enlargement of the TZ. The presence of nodular BPH introduces significant variability in TZ weight, reducing the ability of age to predict prostate size. This suggested that a serum marker that correlates with the presence of TZ nodules may be the best predictor of both prostate size and growth potential. In our most recent studies, we found that BPSA

correlates better with TZ volume than does PSA and can predict clinically significant prostate enlargement better than PSA or fPSA. Using linear regression models, we found that BPSA and fPSA have a log-linear relation to prostate volume and TZ volume; however, unlike that of PSA, the relation of BPSA and fPSA to TZ and total prostate volumes is independent of age.

Diagnostic Utility of BPSA

Because tPSA, fPSA, and BPSA are all mathematically and statistically correlated with total prostate and TZ volume, we sought to determine whether serum assays for any of these forms of the antigen could provide a clinically useful prediction of TZ volume. We plotted ROC curves for each of the 3 serum tests and calculated specificities at 95%, 90%, 85%, and 80% sensitivities for 3 different TZ sizes. The specificity of BPSA for the prediction of TZ enlargement at all sensitivity levels was better than that of PSA. Only BPSA demonstrated a statistically significant ($P < .05$) difference in the area under the curve compared with PSA for a TZ volume of greater than 30 mL.

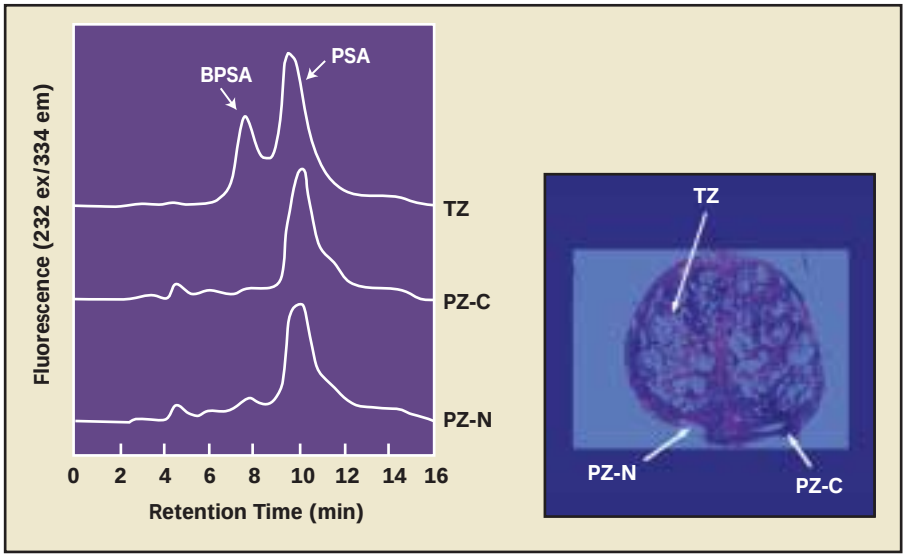

Figure 3. *Benign prostate-specific antigen (BPSA) as a putative benign prostatic hyperplasia. PSA, prostate-specific antigen; TZ, transition zone; PZ-N, peripheral zone-noncancer; PZ-C, peripheral zone-cancer.*

The specificity of BPSA for the prediction of TZ enlargement at all sensitivity levels was better than that of PSA.

Future Directions

Preliminary studies have provided intriguing insight into the biologic basis for the performance of fPSA as a marker to discriminate BPH from prostate cancer and into the molecular forms of PSA driving the relationship between serum PSA levels and TZ and total prostate volumes. However, although the discovery of a serum marker that could establish prostate volume more reliably than a digital rectal examination and avoid the cost and invasiveness of transrectal ultrasound would be a significant advance, our goal is to develop a serum marker for BPH that can provide more insightful diagnosis and management of this disease. The Steering Committee of the Medical Therapy of Prostatic Symptoms (MTOPS) Prostate Samples Analysis Consortium, a National Institutes of Health–funded group of investigators utilizing the specimens and data from the MTOPS trial to develop novel markers for BPH and novel therapeutic targets for BPH therapy, recently delineated the following desired characteristics of any potential novel serum marker for BPH:

1. Determination at the time of initial therapy as to whether the future risk of BPH clinical progression, as defined in MTOPS, justifies the addition of finasteride to the usual practice of initiating α-blocker monotherapy
2. Prediction of future BPH progression
   a. Prediction of symptom progression
   b. Prediction of acute urinary retention/BPH invasive surgery
3. Prediction of response to BPH medical therapy
   a. Prediction of reduction in American Urological Association (AUA) symptom score
   b. Prediction of improvement in maximum urinary flow rate
4. Prediction of future prostate growth

We are currently evaluating the performance of BPSA as a novel serum marker for BPH by these criteria. Our approach has been to develop nomograms that can predict BPH progression using standard clinical predictors. An example of such a nomogram, constructed using data from the phase 3 dutasteride trials, was presented by our group at the AUA 2003 annual meeting.[20] The following predictors at baseline were included in the final nomogram: AUA symptom score, BPH impact index, prior use of α-blockers, PSA level, prostate volume, maximum urinary

flow rate, and randomization group (dutasteride or placebo).

We have begun work developing similar nomograms using data from the MTOPS trial and the Proscar Long-Term Efficacy and Safety Study (PLESS). Our goal is to measure BPSA levels in frozen, archived serum specimens from patients in the dutasteride phase 3 trials, the MTOPS trial, and PLESS and then to compare the predictive accuracy of base nomograms with those that include baseline levels of BPSA. If BPSA represents a clinically important new marker for BPH, we should see improved performance and accuracy of nomogram models that include BPSA level as a predictive parameter.

Nomograms that predict prostate cancer progression were recently demonstrated to achieve a clinically and statistically improved performance compared with base nomogram models when the novel tumor markers transforming growth factor-ß and interleukin-6 soluble receptor were included.[21] The highly anticipated results of these ongoing studies should define the clinical importance of the utility of BPSA in the diagnosis and management of BPH. ■

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Main Points

- Basic research on prostate-specific antigen (PSA) has been a paradigm for the "bench-to-bedside" notion of translational research—that is, basic laboratory research that is quickly "translated" into clinically relevant changes in patient care.
- Recent studies using serum assays specific for various molecular forms of free PSA (fPSA) have demonstrated that the majority of fPSA in the blood is composed of BPSA (benign PSA), truncated forms of proPSA, and an additional form of intact yet inactive PSA.
- In our most recent studies, we found that BPSA correlates better with transition zone (TZ) volume than does PSA and can predict clinically significant prostate enlargement better than PSA or fPSA. BPSA and fPSA have a log-linear relation to prostate volume and TZ volume but, unlike that of PSA, the relation of BPSA and fPSA to total prostate and TZ volumes is independent of age.
- We plotted receiver operator characteristic curves for total PSA, fPSA, and BPSA serum tests and calculated specificities at 95%, 90%, 85%, and 80% sensitivities for 3 different TZ sizes. The specificity of BPSA for the prediction of TZ enlargement at all sensitivity levels was better than that of PSA. Only BPSA demonstrated a statistically significant ($P < .05$) difference in the area under the curve compared with PSA for a TZ volume of greater than 30 mL.
- The ultimate goal is to develop a serum marker for benign prostatic hyperplasia that can provide more insightful diagnosis and management of this disease.